INFECTION AND IMMUNITY, Aug. 1983, p. 636–643
0019-9567/83/080636-08$02.00/0

Vol. 41, No. 2

Inhibition of Macromolecular Synthesis in Cultured Rabbit Cells by *Treponema pallidum* (Nichols)

G. H. W. WONG,†* B. M. STEINER,‡ AND S. GRAVES§

Department of Microbiology, Monash University Medical School, Prahran, Victoria 3181, Australia

Received 25 January 1983/Accepted 23 May 1983

Treponema pallidum partially inhibited the synthesis of DNA, RNA, and protein by rabbit cells in vitro. The inhibition of DNA synthesis was proportional to treponemal concentration and persisted during the period of exposure to *T. pallidum*. The toxic effect was not dependent on treponemal metabolism or on whole treponemes, since heat- and penicillin-killed treponemes and a cell-free sonicate of treponemes had similar toxicities. The toxic factor(s) was also detected in extracts of syphilitic rabbit testes but not in extracts of normal rabbit testes or testes inflamed by chemical means. The *T. pallidum*-derived toxic material had a molecular weight greater than 20,000 as determined by dialysis. Protein and DNA synthesis were most rapidly inhibited; RNA synthesis continued at normal rates for up to 2 h after exposure to treponemes. Protein synthesis or a necessary precursor of protein synthesis appeared to be the primary target of the *T. pallidum* toxin(s).

The mechanism of pathogenesis of *Treponema pallidum* in syphilis is currently unknown. *T. pallidum* is assumed to have little direct toxic effect on host tissues. The tissue destruction in primary syphilis and the serious systemic complications of tertiary syphilis are generally thought to be mediated by host immune responses (12).

Fitzgerald et al. (10) observed that only virulent *T. pallidum* were able to attach to cultured mammalian cells. Heat-killed or nonpathogenic cultivable treponemes did not do so. They suggested that attachment might be related to the pathogenicity of *T. pallidum*. *T. pallidum* attaches by one or both ends to the surface of cultured cells, which are still apparently intact after treponemal infection (11, 20). Thus, attachment of *T. pallidum* to cultured cells does not cause serious observable morphological damage to cells in vitro (11). Recently, however, it has been shown that heavy infection with *T. pallidum* (10^8 per ml) causes dysfunction of nerve cells (27, 31) and morphological damage to a variety of cells in culture (15).

No exotoxins, endotoxins, or other forms of toxins have been detected to date in *T. pallidum* (12). In this report, we demonstrate that a toxic factor (or factors) from *T. pallidum* and syphilitic rabbit testes partially inhibits macromolecular synthesis in mammalian cells in vitro.

MATERIALS AND METHODS

Maintenance of *T. pallidum*. Adult male rabbits were used for the maintenance and passage of *T. pallidum*. After intratesticular inoculation with 5×10^7 treponemes per testis, an orchitis usually developed within 9 to 11 days. The rabbits were sacrificed by intravenous injection of 3 ml of sodium pentobarbitone (200 mg/ml). The testes were aseptically removed and cut into longitudinal and transverse slices with sharp scissors in a petri dish in a laminar flow cabinet. *T. pallidum* cells were extracted anaerobically as previously described (39). The medium used was Eagle minimal essential medium (EMEM) supplemented with 10% fetal calf serum (FCS) with 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid). The pH of the medium was adjusted to 7.4 ± 0.1 with 10 M NaOH. Carbon dioxide and sodium bicarbonate were not used since they had no enhancing effect on treponemal survival in cell-free or tissue culture systems (38).

Extraction of rabbit testes and preparation of high concentrations of *T. pallidum*. A treponemal suspension freshly obtained from orchitic rabbit testes was centrifuged at 1,000 × *g* for 10 min to remove rabbit testicular cells and erythrocytes. The concentration of *T. pallidum* in the supernatant was determined by using a bacterial counting chamber and dark-field microscopy. The supernatant was recentrifuged at 12,000 × *g* for 30 min to pellet the treponemes, which were then diluted with fresh medium to give the appropriate concentrations. The supernatant was the extract of syphilitic rabbit testes. Extracts of normal

† Present address: The Walter and Eiiza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria 3050, Australia.

‡ Present address: Department of Pathology and Laboratory Medicine, Medical School, The University of Texas Health Science Center at Houston, Houston, TX 77025.

§ Present address: Department of Microbiology, LaTrobe University, Bundoora, Victoria 3083, Australia.

rabbit testes or chemically inflamed testes were obtained in the same manner. Chemical inflammation of rabbit testes was induced by intratesticular injection of 0.5 ml of either Freund complete adjuvant or thioglycolate broth (BBL Microbiology Systems, Cockeysville, Md.).

Isolation and maintenance of tissue culture cells. Primary cell cultures were isolated from the tissue of a baby rabbit genital organ (BRGO) or from the testis tissue of an adult rabbit. The tissue was cut and minced into small fragments which were stirred in phosphate-buffered saline containing 0.025% trypsin (Sigma Chemical Co., St. Louis, Mo.) and 100 U of penicillin and 100 μg of streptomycin (Glaxo Laboratories, Ltd., Greenford, Middlesex, England) per ml at 37°C for 30 min. The first extract (25 ml) was discarded. The process was repeated and the second extract (25 ml) was collected and centrifuged at 1,000 × *g* for 10 min to pellet the cells, which were then resuspended in HEPES-EMEM with 20% heat-inactivated FCS containing antibiotics (as above). Subsequently, the cells were grown without antibiotics at 37°C, and the medium was changed every 3 days. After the first two passages, the cells were grown in HEPES-EMEM with 10% FCS. The confluent monolayers were subcultured with phosphate-buffered saline containing 0.025% trypsin and 0.001% EDTA (Sigma).

The C76/203 (human epithelial tumor) and BHK (baby hamster kidney) cell lines are established cell lines which were the generous gifts of E. A. V. Pihl and Keith Stuckly (both at Monash University Prahran, Victoria, Australia), respectively. These cells were all passaged in EMEM-HEPES with 10% FCS.

Determination of [^{3}H]thymidine, [^{3}H]uridine, and [^{3}H]leucine incorporation by mammalian cells. Freshly trypsinized mammalian cells were dispensed into each well of a 96-well microtiter plate (Sterilin, England) and allowed to attach for at least 12 h. The attached cells were washed twice with medium before experimental use. Experimental samples (200 μl) were then added to each well. After a defined period of aerobic coincubation at 37°C, 0.5 μCi of [^{3}H]thymidine (20 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) was added to each well. The supernatant was discarded at the end of the labeling period, and the mammalian cells were solubilized with 150 μl of lysis buffer (0.5 M NaOH, 0.05% sodium dodecyl sulfate). Using a multiple-cell culture harvester (Skatron, Norway), we collected the trichloroacetic acid (TCA) precipitates onto glass fiber filters (Flow Laboratories, McLean, Va.). Radioactivity was determined with a Packard Tri-carb scintillation counter, using 5 g of 2,5-diphenyloxazole (PPO) per liter and 0.4 g of 1,4-bis[2 (4-methyl-5-phenyloxazolyl)]-benzene (dimethyl-POPOP) per liter in toluene as the scintillation cocktail. Data were compared by means of analysis of variance, and the differences between the compared groups were considered significant when $P < 0.05$. Percent inhibition was calculated according to the formula:

(1 - ^{3}H isotope incorporated by infected cells)/

(^{3}H isotope incorporated by uninfected cells) × 100

After RNA and protein labeling, the mammalian cells were solubilized with 150 μl of a mixture of trypsin (0.05%) and EDTA (0.03%) in phosphate-buffered saline instead of the lysis buffer used for DNA labeling. Since viable *T. pallidum* cells have the ability to incorporate [^{3}H]uridine (26) and [^{3}H]leucine (1) into RNA and protein, respectively, heat-killed *T. pallidum* cells were used in the experiments measuring the inhibition of RNA and protein synthesis in BRGO cells. Experiments in our laboratory had shown that viable and heat-killed *T. pallidum* cells are equally inhibitory for DNA synthesis in BRGO cells. Counting and harvesting of cells and the determination of percentage inhibition were all carried out as for DNA.

Dialysis of toxic extracts. To determine whether the toxic components of a *T. pallidum* sonicate and the syphilitic testes extracts were of high or low molecular weight, we dialyzed them. Dialysis tubing was washed in distilled water and boiled for 10 min against three changes of 1 mM EDTA to remove any toxic materials which might be associated with the tubing. Samples of the extracts (5 ml) were dialyzed against 1 liter of EMEM overnight at 4°C. The samples were then resterilized by filtration (0.45-μm pore size; Millipore Corp., Bedford, Mass.). Normal rabbit testes extract was used as a negative control to determine whether the dialysis procedure itself produced any toxicity for BRGO cells.

RESULTS

Effect of DTT on DNA synthesis by BRGO cells. Since *T. pallidum* is very sensitive to oxygen (39), reducing agents are generally used to prolong its survival in vitro in both cell-free and tissue culture systems (14). To determine whether reducing agents could be used in experiments in which *T. pallidum* toxicity for mammalian cells was being determined, we examined the effect of dithiothreitol (DDT) on BRGO cell DNA synthesis. BRGO cells were exposed to 0.5 mM DTT for 4 h in 96-well microtiter trays, and then 0.5 μCi of [^{3}H]thymidine was added per well. BRGO cells exposed to DTT incorporated 542 ± 189 cpm per well, whereas control cultures incorporated 1,215 ± 109 cpm per well (a 55% inhibition of DNA synthesis). Peterkofsky and Prather (30) also observed that DTT (0.2 mM) inhibited the growth of chick embryo fibroblasts by 52% over 24 h although they found that other reducing agents such as ascorbate, glutathione, or cysteine were more toxic to tissue culture cells than DTT.

Similarly, glutathione, cysteine, and DTT inhibit the response of nerve cells in culture to electrical stimulation (28). Thus, in our subsequent experiments, reducing agents were not used so as to eliminate their toxic effect on BRGO cells.

***T. pallidum* toxicity for DNA synthesis in BRGO cells.** Intact BRGO cell monolayers were incubated with various concentrations of viable treponemes (10^3 to 10^{10} per ml), and the effect on DNA synthesis in BRGO cells were measured. The incorporation of [^{3}H]thymidine by *T. pallidum*-infected BRGO cells was lower than in the uninfected control, and the inhibition was dose

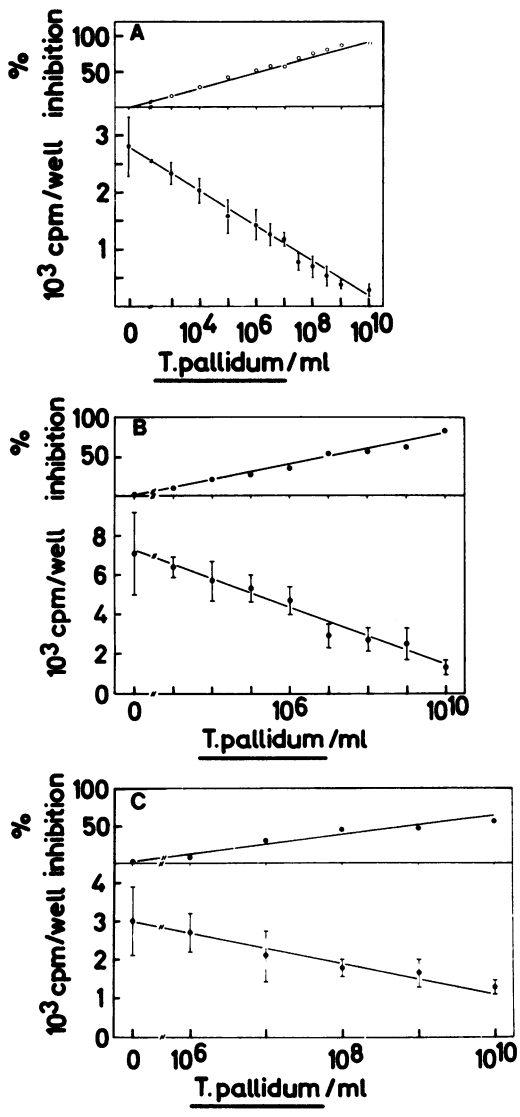

FIG. 1. Inhibition of [^{3}H]thymidine (A), [^{3}H]uridine (B), and [^{3}H]leucine (C) incorporation in BRGO cells by different concentrations of viable (A) or heat-killed (B and C) *T. pallidum*. Treponemes were concentrated by high-speed centrifugation and diluted to give different concentrations. In the experiments to determine the inhibition of RNA (B) or protein (C) synthesis in BRGO cells, the treponemes were killed by heating to 56°C for 10 min before use. A 200-μl portion of treponeme suspension (10^3 to 10^{10} per ml) were added to 10^3 BRGO cells per well. After 4 h of coincubation, 0.5 μCi of either [^{3}H]thymidine (A) [^{3}H]uridine (B), or [^{3}H]leucine (C) was added to each well. BRGO cells were allowed to incorporate the labeled compound for 24 h. The cells were then solubilized, the TCA-precipitates were collected and radioactivity was determined. Each point is the mean of 5 (A) or 15 (B and C) samples ± standard deviations.

dependent (Fig. 1A). Since *T. pallidum* has no thymidine kinase (2, 27), the incorporation of [^{3}H]thymidine by *T. pallidum* was found to be minimal (28 ± 17 cpm for treponemes without tissue cells). Coincubation with 10^8 treponemes per ml resulted in a 75% inhibition in DNA synthesis by BRGO cells. This was the same concentration of *T. pallidum* which caused the dysfunction of nerve cells (28, 31) and morphological damage in a variety of cells (15) in vitro. When the concentration of treponemes was 10^{10} per ml, the inhibition was 90% (Fig. 1A). Similar results were obtained when the toxic effect of *T. pallidum* was tested on a variety of other cell types. Other tissue culture cells tested were human skin tumor cells (C76/203), a primary rabbit testes cell cultures, and BHK cells (data not shown). Although all cells showed sensitivity to *T. pallidum* toxicity, the response was not identical with all cell types. The BHK cells became damaged and vacuolated within a few hours of exposure to *T. pallidum*, whereas human skin tumor C76/203 cells were more resistant and showed far less damage. The primary rabbit testes and BRGO cell cultures were virtually identical in response to the toxic activity of *T. pallidum*. The BRGO cell culture was chosen for further study of *T. pallidum* for two reasons: (i) it was derived from a susceptible host for *T. pallidum* and thus could be related most easily to in vivo pathology and (ii) it is capable of sustaining prolonged in vitro survival of *T. pallidum* (37) and is most similar to the cell type used in cocultivation work (9).

The time course of incorporation of [^{3}H]thymidine by BRGO cells after infection by viable *T. pallidum* was studied. DNA synthesis continued in cells infected with 10^8 treponemes per ml but as a greatly reduced rate compared to the uninfected control (Fig. 2). After coincubation for 1 h, DNA synthesis was inhibited by 75% in infected cells, indicating that the inhibitory process was rapid. The viability of treponemes began to decline after 4 to 5 h under aerobiosis, even in the presence of tissue culture cells. However, the rate of inhibition was constant over the course of the experiment (Fig. 2), suggesting that the toxic factor is relatively stable and not dependent on treponemal survival. The toxic effect was followed for periods beyond 24 h in some experiments. Morphological changes and detachment of cells were seen after 3 days when BRGO cells were exposed to 10^8 treponemes or greater (data not shown). After approximately 5 days of exposure, the cell sheet had almost completely detached, whereas control cells were still normal in appearance. The disruption of mammalian cells in tissue culture as measured by morphological alterations and degradation has been observed by others (15, 28). Labeling

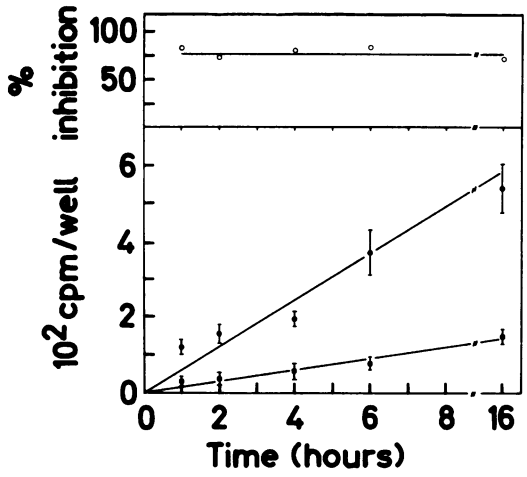

FIG. 2. Time course of the incorporation of [^{3}H]thymidine by normal (●) and infected (■) BRGO cells. A 200-μl portion of viable treponemes (10^8 per ml) and 0.5 μCi of [^{3}H]thymidine were added to 10^3 BRGO cells in each well. After fixed periods of coincubation, BRGO cells were solubilized, TCA were precipitates collected, and radioactivity was determined. Each point is the mean of five samples ± standard deviation.

studies were not normally continued beyond 24 h as nonlinear incorporation over longer periods occurred in control cultures, indicating a possible radomizing of label which would not have allowed for valid comparisons.

Effect of *T. pallidum* on synthesis of protein and RNA in BRGO cells. To determine whether *T. pallidum* had any effect on the synthesis of macromolecules other than DNA, BRGO cells were labeled with either [^{3}H]leucine (protein synthesis) or [^{3}H]uridine (RNA synthesis) in the presence of *T. pallidum* killed by heating at 56°C for 10 min. Preliminary experiments showed that heat-killed *T. pallidum* did not incorporate leucine or uridine into TCA-precipitable material (data not shown). BRGO cells exposed to heat-killed *T. pallidum* showed a dose-dependent inhibition of RNA synthesis (Fig. 1B) and protein synthesis (Fig. 1C) similar to that seen with DNA synthesis (Fig. 1A). Inhibition of RNA and protein synthesis was not as complete as with DNA synthesis; e.g., at 10^8 per ml RNA synthesis was inhibited by 62% and protein synthesis by 50% compared to a 75% inhibition of DNA synthesis by the same number of treponemes.

Demonstration of toxic activity in nonviable *T. pallidum*. To test whether toxicity was dependent on treponemal viability, *T. pallidum* (10^8 per ml) were inactivated by several methods. Heat-killed or penicillin-treated suspensions of treponemes or a membrane-filtered sonicate of treponemes (all prepared at the same initial concentrations of *T. pallidum*) had similar inhibitory effects (Fig. 3). When compared to the control, DNA synthesis in BRGO cells treated with inactivated *T. pallidum* or with viable *T. pallidum* were all inhibited by approximately 80%. Viable treponemes were not significantly more toxic than inactivated treponemes. Since the sonicate contained no intact treponemes but only soluble components and presumably some membrane vesicles, this toxic effect can probably not be attributed to treponemal metabolism. This result suggested that the inhibition of DNA synthesis by BRGO cells was not dependent on the attachment of viable treponemes to cultured cells but was mediated by one or more toxic factors associated with the treponemes.

Demonstration of toxic activity in extracts of syphilitic rabbit testes. The toxic component(s) was also detected in the cell-free extracts of syphilitic rabbit testes. Extracts of normal rabbit testes or of chemically inflamed rabbit testes did not show any inhibitory effect on DNA synthesis in BRGO cells. Extracts of normal rabbit testes enhanced DNA synthesis in BRGO cells by 60%, extracts from chemically-inflamed testes showed only slightly less enhancement of DNA synthesis in BRGO cells than the extracts of

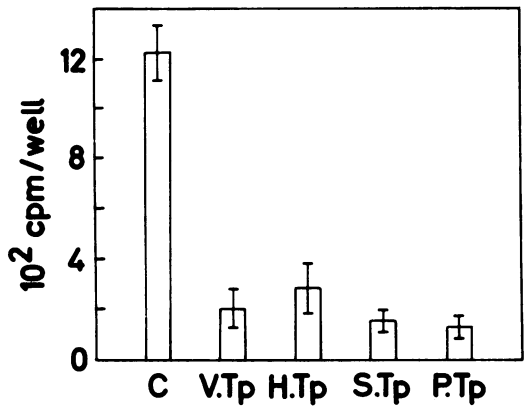

FIG. 3. Effect of heat- or penicillin-killed treponemes or of a sonicated cell-free extract of *T. pallidum* on the incorporation of [^{3}H]thymidine in BRGO cells. Freshly harvested treponemes (10^8 per ml) were heat killed (56°C, 10 min), penicillin treated (100 U/ml, 2 h), or sonicated (15 amplitude, 2 min; Soniprep MSE ultrasonic disintegrator). The sonicated suspension was filtered (0.45 μm pore size; Millipore) to remove any bacteria which may have contaminated the suspension during sonication. A 200-μl portion of the viable or killed treponemes or of the treponemal extract was added to 10^3 BRGO cells per well. Radiolabeling was performed as described in the legend to Fig. 1. Each bar represents the mean of 10 samples ± standard deviation. Abbreviations: V.Tp, viable *T. pallidum*; H.Tp, heated *T. pallidum*; S.Tp, sonicated *T. pallidum*; P.Tp, penicillin-treated *T. pallidum*.

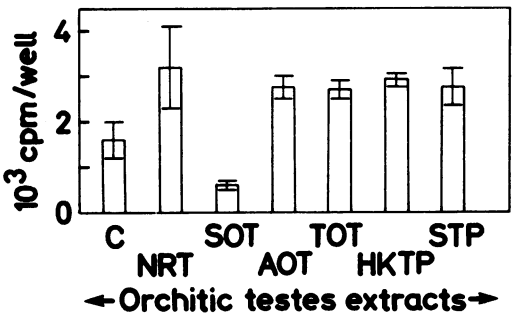

FIG. 4. Effect of testes extracts on DNA synthesis in BRGO cells. Extracts were made from either normal rabbit testes or treated rabbit testes 11 to 14 days after injection with 5×10^7 viable, heat-killed or sonicated treponemes per testis. Sterile inflammation was induced in testes by injecting each testis with 0.5 ml of either Freund complete adjuvant or thioglycolate medium. The extracts were made as soon as inflammation became obvious. Extracts of these rabbit testes were the high-speed supernatants described in the text. BRGO cells (ca. 10^4 cells per well) were treated with these testes extracts for 24 h before radiolabeling. EMEM medium with 10% FCS and 10 mM HEPES was used. Abbreviations: NRT; normal rabbit testes extract; SOT, syphilitic orchitic rabbit testes extract; AOT, adjuvant-induced orchitic rabbit testes extract; TOT; thioglycolate-induced orchitic rabbit testes extract; HKTP; rabbit testes sham infected with 5×10^7 heat-killed *T. pallidum*, harvested and extracted 11 days after injection; STP, rabbit testes sham infected with the sonicated extract of 5×10^7 *T. pallidum* and harvested 11 days after injection

normal testes (Fig. 4). In contrast, extracts from syphilitic orchitic testes depressed DNA synthesis in BRGO cells by more than 70% (Fig. 4).

To eliminate any potential inhibitory factor(s) present in the orchitic testicular extracts, treponemes were washed in phosphate-buffered saline and suspended in fresh medium. A membran-filtered sonicate of these treponemes (10^8 per ml) was significantly more toxic ($P < 0.05$) than the extract of syphilitic orchitic rabbit testes from which they were derived (Fig. 5). This result suggests that the toxic factor(s) is associated with *T. pallidum*. To determine whether the material present in the syphilitic rabbit testes extract and the extract of *T. pallidum* was of large molecular weight, they were dialyzed overnight against EMEM without serum. In neither case was the toxicity reduced by dialysis, indicating that the toxic principle in each extract had a molecular weight greater than the 20,000 exclusion limit of the dialysis tubing (Fig. 6).

Time course of inhibition of DNA, RNA, and protein synthesis in BRGO cells due to toxin(s) from heat-killed *T. pallidum*. Protein synthesis continued in BRGO cells with 10^8 *T. pallidum* per ml but at a reduced rate when compared to the untreated control (Fig. 7A). The inhibition was approximately 50% after only 15 min of exposure to *T. pallidum* but did not increase beyond this level within the experimental period. Inhibition of DNA synthesis was slower, reaching a level of 60% 45 min after exposure to *T. pallidum* (10^8 per ml) (Fig. 7B). RNA synthesis was the least affected by *T. pallidum* since no significant difference could be detected over the first 90 min of exposure (data not shown). Only when the exposure was continued for longer periods was a significant inhibition detected, being 50% at 4 h and constant thereafter (Fig. 7C). These results suggest that protein synthesis may be the primary target of action of the treponemal toxin(s).

DISCUSSION

Pathogenesis in many bacterial infections involves the production of toxin(s) by the invading bacteria. Toxins may be directly responsible for the disease as found in diphtheria (7), bacterial dysentery (19), and cholera (34), or they may simply contribute to the pathogenic process. In the latter case, a wide variety of enzymes, metabolites, and structural components of the cell may be involved.

In syphilis, the reasons for the characteristic

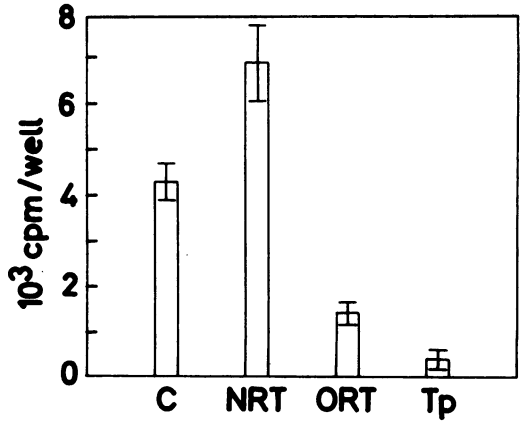

FIG. 5. Effect of extracts from normal or syphilitic rabbit testes or *T. pallidum* (Tp) extract on incorporation of [^{3}H]thymidine by BRGO cells. The extracts of normal rabbit testes (NRT) and orchitic rabbit testes (ORT) were the supernatants obtained by high-speed centrifugation as described in the text. The *T. pallidum* extract (10^8 per ml) was made by sonication and sterilized by filtration. The control was EMEM supplemented with 10% FCS and 10 mM HEPES. Portions (200-μl) of the above extracts were added to 10^3 BRGO cells per well. Radiolabeling was performed as described in the legend to Fig. 1. Each bar represents a mean of 10 samples ± standard deviations.

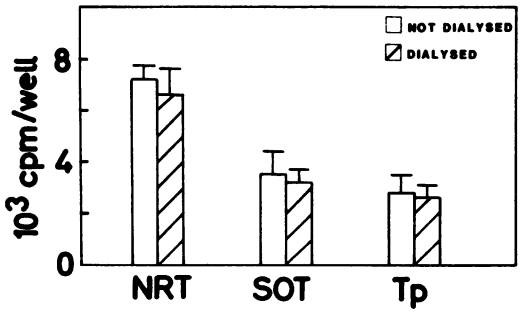

FIG. 6. Effect of dialysis on the toxicity of *T. pallidum* or syphilitic orchitic testes extracts toward DNA synthesis in BRGO cells. Extracts of normal rabbit testes (NRT), syphilitic orchitic rabbit testes (SOT), and *T. pallidum* (Tp) were dialyzed for 24 h against EMEM medium without serum at 4°C. BRGO cells were treated with extracts with or without dialysis for 24 h before radiolabeling. Each bar represents the mean of 15 samples ± standard deviations.

pathology seen in the various stages of the disease are unknown. Lesion development involves the breakdown of vascular tissue and the ground substance of connective tissue (32). As the immune response develops, further tissue damage occurs, leading to ulceration (22). *T. pallidum* appears to breakdown connective tissue (32, 33), possibly by means of a mucopolysaccharidase (13, 32). The reason for the damage to a variety of target organs that occurs in tertiary syphilis is less clear. No good explanation has been offered for these later pathological changes, although autoantibodies to heart tissue have been reported (6), indicating that at least part of the damage may be mediated by autoimmune phenomena.

What part the bacterium itself directly plays in the various pathological changes observed in the course of the disease is unknown. No exotoxins, endotoxins, or other treponemal toxins have been conclusively demonstrated (12). Recently, however, it has been shown that heavy infection of tissue culture cells results in morphological destruction of the infected cells (15) and dysfunction of nerve cells in vitro (28, 31). The concentration used by Fitzgerald et al. (15), Oakes et al. (28), and Repesh et al. (31) (10^8 *T. pallidum* per ml) was that which we have shown inhibits DNA synthesis by 75% (Fig. 1A), RNA synthesis by 62% (Fig. 1B), and protein synthesis by 40% (Fig. 1C) in BRGO cells in culture. This dose-dependent inhibition is unlikely to be attributable to treponemal metabolism or competition for a nutrient since a cell-free extract of *T. pallidum* prepared by sonication was just as inhibitory as viable treponemes (Fig. 3).

Time course experiments indicated that protein synthesis was affected most rapidly, with DNA synthesis being impaired shortly afterward (Fig. 7A and B). RNA synthesis was not affected until much later and did not appear to be the primary target of the toxin. The inhibition of RNA synthesis, detected after longer periods (4 h) of exposure to treponemal toxin (Fig. 7C),

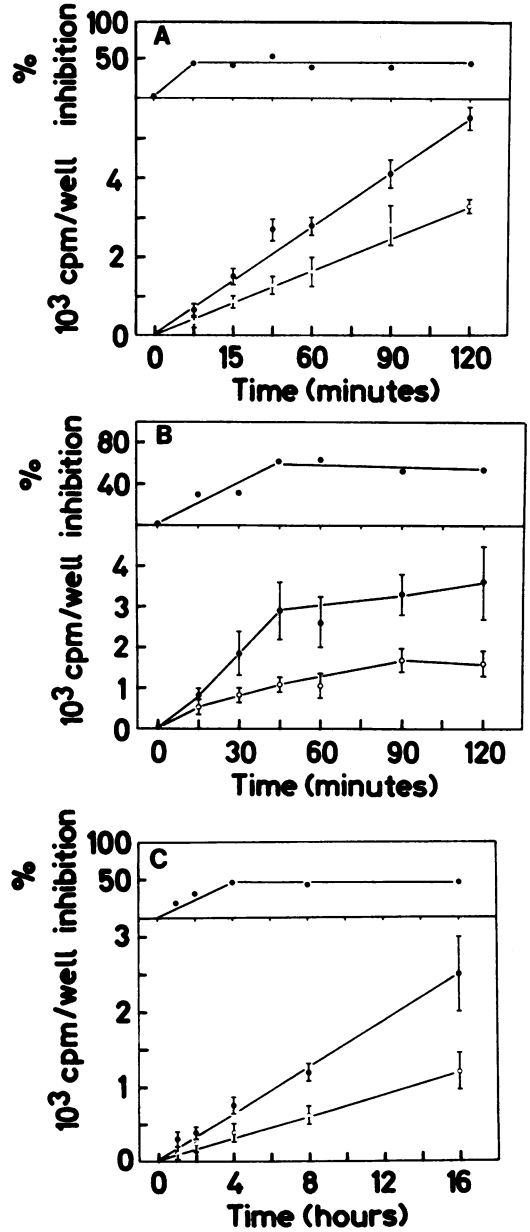

FIG. 7. Time course of incorporation of [^{3}H]leucine (A), [^{3}H]thymidine (B), and [^{3}H]uridine (C), by normal BRGO cells (●) and BRGO cells treated with heat-killed *T. pallidum* (10^8 per ml) (○). Each point is the mean of 15 samples ± standard deviation in three separate experiments.

probably resulted from initial inhibition of protein (and perhaps DNA) synthesis. Similar inhibition of protein and DNA synthesis has been observed for *Shigella* toxin (4), abrin (24), ricin (25), and emetine (18), with recent work indicating that ricin inhibits DNA polymerase (3). The inhibition of protein synthesis reached maximum level within 15 min (Fig. 7A), which was the earliest time tested experimentally. It is possible that the inhibitory effect might be due to an inhibition of utilization of exogenous amino acids, a depletion of intracellular amino acid pools, an inhibition of cellular energy metabolism, or a partial block in protein synthesis. The inhibition of DNA synthesis may be a consequence of inhibition of protein synthesis since it occurred 30 min later (compare Fig. 7A and B), perhaps after the failure to synthesize or accumulate a sufficient concentration of initiation proteins which may be necessary for initiation of DNA synthesis (29). Since macromolecular synthesis did not stop completely, the effect of the toxin(s) was probably only cytostatic. This may also be due to a decrease in the population of cells incorporating label, a partial block of the cells in one stage of the cell cycle, or both. Thus, the cells could continue to synthesize macromolecules, but at a reduced rate compared to the untreated controls.

This toxic substance was found free in the fluid which accumulated in the syphilitic orchitic rabbit testes in response to infection with *T. pallidum* (Fig. 4). In contrast, chemically inflamed orchitic rabbit testes contained no such inhibitory substance. Cell-free extracts prepared from washed *T. pallidum* were significantly more toxic than the testicular extract from which they were prepared (Fig. 5). These results support the idea that the toxic material is derived from the treponemes and not the host, and its activity in cell-free extracts of *T. pallidum* suggest that the effect is via a direct toxic action on the host cell rather than by a simple competition between treponeme and host cell for a nutrient substance.

The importance of this toxic substance will be better understood once the chemical nature of this material is known. The possibility exists that the toxic activity resides in a structural component of *T. pallidum*. The inhibitory extracts and the *T. pallidum* sonicate all showed an endotoxin-like activity as assessed by the *Limulus* lysate assay (unpublished data). Because tissue destruction is proceeding by the time of harvest and both polynucleotides and proteins can interfere with the *Limulus* lysate test under certain conditions (8), this result is only suggestive. The Jarisch-Herxheimer reaction, which commonly occurs after antibiotic treatment of patients with syphilis (5), may be caused by a treponemal endotoxin-like molecule (16). Studies with nonpathogenic treponemes might also be helpful since *Treponema phagedenis* is known to contain a lipopolysaccharide-like material (21). Whether this material is toxic for cultured mammalian cells is presently unknown. In other systems, endotoxin has been shown to directly effect mammalian cells in organ culture (17). The possibility of an intracellular residence of *T. pallidum* at times during the infection (35, 36) also may relate to this toxicity for mammalian cells. *Shigella dysenteriae* toxin has been shown to cause many of its effects after the bacterium has penetrated the host cell (19). An exact determination of these mechanisms must await the isolation of the toxin molecule; since this in turn is dependent on the availability of large quantities of treponemes with minimal host contamination, it would be greatly facilitated by an in vitro cultivation system.

ACKNOWLEDGMENTS

This work was supported by grants from the Austrialian National Health and Medical Research Council, the Utah Foundation, the Heiser Program for Leprosy Research, the Australian-Britian Society, and Monash University, from whom funds are gratefully acknowledged.

We thank S. Faine for use of the facilities of the Monash University Microbiology Department.

LITERATURE CITED

1. **Baseman, J. B., and N. Hayes.** 1974. Protein synthesis by *Treponema pallidum* extracted from infected rabbit tissue. Infect. Immun. **10**:1350–1355.
2. **Baseman, J. B., J. C. Nichols, and S. Mogerley.** 1979. Capacity of virulent *Treponema pallidum* (Nichols) for deoxyribonucleic acid synthesis. Infect. Immun. **23**:392–397.
3. **Bhattacharya, P., I. Simet, and S. Basu.** 1979. Inhibition of human neuroblastoma DNA polymerase activities by plant lectins and toxins. Proc. Natl. Acad. Sci. U.S.A. **76**:2218–2221.
4. **Brown, J. E., S. W. Rothman, and B. P. Doctor.** 1980. Inhibition of protein synthesis in intact HeLa cells by *Shigella dysenteriae* I toxin. Infect. Immun. **29**:98–107.
5. **Bryceson, A. D. M.** 1976. Clinical pathology of the Jarisch-Herxheimer reaction. J. Infect. Dis. **133**:696–704.
6. **Casavant, C. H., V. Wicher, and K. Wicher.** 1978. Host response to *Treponema pallidum* III. Demonstration of autoantibodies to heart in sera from infected rabbits. Int. Arch. Allergy Appl. Immunol. **56**:171–178.
7. **Collier, R. J.** 1975. Diphtheria toxin: mode of action and structure. Bacteriol. Rev. **39**:54–85.
8. **Elin, R. J., and S. M. Wolff.** 1973. Nonspecificity of the Limulus amoebocyte lysate test: positive reactions with polynucleotides and proteins. J. Infect. Dis. **128**:349–352.
9. **Fieldsteel, A. H., D. L. Cox, and R. A. Moeckli.** 1981. Cultivation of virulent *Treponema pallidum* in tissue culture. Infect. Immun. **32**:908–915.
10. **Fitzgerald, T. J., R. C. Johnson, J. N. Miller, and J. A. Sykes.** 1977. Characterization of the attachment of *Treponema pallidum* (Nichols strain) to cultured mammalian cells and the potential relationship of attachment to pathogenicity. Infect. Immun. **18**:467–478.
11. **Fitzgerald, T. J., P. Cleveland, R. C. Johnson, J. N. Miller, and J. A. Sykes.** 1977. Scanning electron microscopy of *Treponema pallidum* (Nichols strain) attached to cultured mammalian cells. J. Bacteriol. **130**:1333–1344.

12. **Fitzgerald, T. J.** 1981. Pathogenesis and immunology of *Treponema pallidum*. Annu. Rev. Microbiol. **35**:29–54.
13. **Fitzgerald, T. J., and R. C. Johnson.** 1979. Mucopolysaccharidase of *Treponema pallidum*. Infect. Immun. **24**:261–268.
14. **Fitzgerald, T. J., R. C. Johnson, and E. T. Wolff.** 1980. Sulphydryl oxidation using procedures and experimental conditions commonly used for *Treponema pallidum*. Br. J. Vener. Dis. **56**:129–136.
15. **Fitzgerald, T. J., L. A. Repesh, and S. G. Oakes.** 1982. Morphologic destruction of cultured cells mediated through the attachment of *Treponema pallidum*. Br. J. Vener. Dis. **58**:1–11.
16. **Gelfand, J. A., R. J. Elin, F. W. Berry, and M. M. Frank.** 1976. Endotoxemia associated with the Jarisch-Herxheimer reaction. N. Engl. J. Med. **295**:211–213.
17. **Gregg, C. R., M. A. Melly, C. G. Hellerquist, J. R. Conigilio, and Z. A. McGee.** 1981. Toxic activity of purified lipopolysaccharide of *Neisseria gonorrhoeae* for human fallopian tube mucosa. J. Infect. Dis. **143**:432–439.
18. **Grollman, A. P.** 1968. Inhibitors of protein synthesis. Effects of emetine on protein and nucleic acid biosynthesis in HeLa cells. J. Biol. Chem. **243**:4089–4094.
19. **Hale, T. L., and S. B. Formal.** 1981. Protein synthesis in HeLa or Henle 407 cells infected with *Shigella dysenteriae* 1, *Shigella flexneri* 2a, or *Salmonella typhimurium* W118. Infect. Immun. **32**:137–144.
20. **Hayes, N. S., K. E. Muse, A. M. Collier, and J. B. Baseman.** 1977. Parasitism of virulent *Treponema pallidum* of host cell surfaces. Infect. Immun. **17**:174–186.
21. **Jackson, S. W., and P. N. Zey.** 1973. Ultrastructure of lipopolysaccharide isolated from *Treponema pallidum*. J. Bacteriol. **114**:838–844.
22. **Knox, J. M., D. Mucher, and N. D. Guzick.** 1976. The pathogenesis of syphilis and the related treponematoses, p. 249–259. *In* R. C. Johnson (ed.), The biology of parasitic spirochaetes. Academic Press, Inc., London.
23. **Levene, G. M., J. L. Turk, D. J. M. Wright, and A. G. S. Grimble.** 1969. Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. Lancet **2**:246–247.
24. **Lin, J. Y., W. Y. Kas, K. Y. Tserng, C. C. Chen, and T. C. Tung.** 1970. Effect of crystalline abrin on the biosynthesis of protein, RNA and DNA in experimental tumors. Cancer Res. **30**:2431–2433.
25. **Lin, J. Y., K. Lin, C. C. Chen, and T. C. Tung.** 1971. Effect of crystalline ricin on the biosynthesis of protein, RNA and DNA in experimental tumors. Cancer Res. **31**:921–924.
26. **Nichols, J. C., and J. B. Baseman.** 1978. Ribosomal ribonucleic acid synthesis by virulent *Treponema pallidum*. Infect. Immun. **19**:854–860.
27. **Norris, S. J., J. N. Miller, and J. A. Sykes.** 1980. Long-term incorporation of tritiated adenine into deoxyribonucleic acid and ribonucleic acid by *Treponema pallidum* (Nichols strain). Infect. Immun. **29**:1040–1049.
28. **Oakes, S. G., L. A. Repesh, R. S. Pozos, and T. J. Fitzgerald.** 1982. Electrophysiological dysfunction and cellular disruption of sensory neurones during incubation with *Treponema pallidum*. Br. J. Vener. Dis. **58**:220–227.
29. **Pardee, A. B., R. Dubrow, J. L. Hamlin, and R. F. Kletzien.** 1978. Animal cell cycle. Annu. Rev. Biochem. **47**:715–750.
30. **Peterkofsky, B., and W. Prather.** 1977. Cytotoxicity of ascorbate and other reducing agents towards cultured fibroblasts as a result of hydrogen peroxide formation. J. Cell. Physiol. **90**:61–70.
31. **Repesh, L. A., T. J. Fitzgerald, S. Oakes, and R. S. Pozos.** 1982. Scanning electron microscopy of the attachment of *Treponema pallidum* to nerve cells *in vitro*. Br. J. Vener. Dis. **58**:211–219.
32. **Scott, V., and G. J. Dammin.** 1950. Hyaluronidase and experimental syphilis III. Metachromasia in syphilitic orchitis and its relationship to hyaluronic acid. Am. J. Syph. **34**:501–514.
33. **Scott, V., and G. J. Dammin.** 1954. Morphological and histochemical sequences in syphilitic and tuberculous orchitis in the rabbit. Am. J. Syph. **38**:189–202.
34. **Sharp, G. W. G.** 1973. Action of cholera toxin on fluid and electrolyte movement in the small intestine. Annu. Rev. Med. **24**:19–28.
35. **Sykes, J. A., and J. N. Miller.** 1971. Intracellular location of *Treponema pallidum* (Nichols strain) in the rabbit testis. Infect. Immun. **4**:307–314.
36. **Sykes, J. A., J. N. Miller, and A. J. Kalan.** 1974. *Treponema pallidum* within cells of primary chancre from a human female. Br. J. Vener. Dis. **50**:40–44.
37. **Wong, G. H. W., B. Steiner, S. Faine, and S. Graves.** 1983. Factors affecting the attachment of *Treponema pallidum* (Nichols) to mammalian cells *in vitro*. Br. J. Vener. Dis. **59**:21–29.
38. **Wong, G. H. W., B. M. Steiner, and S. Graves.** 1982. The lack of effect of bicarbonate on the survival of *Treponema pallidum* (Nichols) *in vitro*. Br. J. Vener. Dis. **58**:130.
39. **Wong, G. H. W., B. M. Steiner, and S. Graves.** 1982. A comparison of anaerobic and microaerophilic conditions of extraction and incubation on the survival of *Treponema pallidum in vitro*. Br. J. Vener. Dis. **58**:139–142.